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Capillary ion chromatography of inorganic anions on octadecyl silica monolith modified with an amphoteric surfactant

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Abstract

A reversed-phase monolithic silica based capillary column (Onyx C_{18}, 150 mm × 0.1 mm) was modified with the amphoteric surfactant, *N*-dodecyl-*N*,*N*-(dimethylammonio)undecanoate (DDMAU) and evaluated for the separation and determination of inorganic anions using on-column capacitively coupled contactless conductivity detection (C^4D). The chromatographic performance of the column was evaluated and under optimal conditions separation efficiencies of 56,200 plates per meter or 7025 plates per column (at detection point) were observed (for iodide). Direct plumbing of the capillary column to the micro-injector and on-column detection eliminated extra-column band broadening, thus allowing accurate analysis of van Deemter curves obtained for the monolithic capillary column. The calculated value for the *C*-term in the obtained van Deemter curve was between 3 and 4 ms for inorganic anions, allowing for the utilisation of relatively high flow rates without significant losses in efficiency. The performance of the C^4D detector was investigated and compared for detection on an open tubular capillary column and on the modified monolithic silica capillary column. The on-column detection approach did not result in any significant decrease in peak sensitivity for the monolith compared to responses recorded for open tubular capillary columns, and in addition meant the system could be applied to rapid separations by simple variation in apparent column length. The proposed chromatographic system allowed for detection of common anions at sub-ppm level with a 10 nL injection volume. Additionally, on-column detection allowed visualisation of the development of the separation at any point in time and evaluation of the longitudinal uniformity of the ion-exchange coating.

Keywords: Capillary ion chromatography; Monolithic silica column; Inorganic anions; Contactless conductivity detection; Drinking water

1. Introduction

Since pioneering work in 1983 [1,2] capillary ion chromatography (CapIC) has generated steady interest due to potential benefits such as low reagent consumption, rapid and efficient separations, analysis of small sample volumes of high matrix complexity, and simple interfacing of CapIC columns with selective and sensitive mass-spectrometric detection. Such advantages are discussed in detail in a recent review of the latest developments and achievements in CapIC, which has been compiled by Kuban and Dasgupta [3]. However, CapIC, as with most microfluidic technologies, is highly dependent upon the quality and robustness of the instrumentation used, and for highly efficient capillary based separations both the quality of the column packing and the elimination of all extra-column band broadening are of great importance.

Three types of capillary columns have been used in CapIC: packed columns, open tubular columns and most recently introduced, monolithic columns. The first publication on CapIC was by Rokushika et al. [1] who used a surface agglomerated anion-exchange capillary column (50 mm × 0.19 mm I.D.), packed with 10 μm particles, coupled to a 0.2 mm × 10 mm nafion perfluorosulphonate hollow fibre suppressor with conductivity detection. Later in the same year, Rokushika et al. used the same column type, albeit much longer (470 mm × 0.19 mm I.D.), for the separation of seven anions in 15 min, in conjunction with UV detection [2]. A longer capillary column of smaller internal diameter (500 mm × 0.18 mm) packed with 13 μm IonPac AS11

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resin particles was used by Boring et al. [4] with suppressed conductivity detection, resulting in the maximum peak efficiency of 27,100 theoretical plates per meter (N/m) calculated for the peak of chloride.

The use of open tubular capillary columns for CapIC have two main disadvantages –low capacity and the subsequent necessity to use rather long narrow coated capillaries to achieve sufficient separation efficiency as compared with packed capillaries. The capacity of open tubular capillaries can be increased either with multilayer organisation of bonded anion-exchange groups or by immobilisation of micro-particles of a suitable anion exchanger. Dasgupta and Kuban reported the use of 5 m × 0.075 mm I.D. open tubular capillary column coated with a 0.7 μm layer of an anion-exchange polymer for the separation of four anions in 35 min [3]. Pyo and Kim [5] used an open tubular capillary column of 1 m × 0.05 mm I.D. with immobilised latex nano-particles of 360 nm diameter. However, such long narrow columns exhibited a high backpressure which necessitated operation at high temperatures, from 75 to 150 °C.

In recent years attention has turned to monolithic capillary columns for all modes of HPLC, including CapIC, although there has only been a limited amount of work presented using pressure-driven flow together with capillary columns containing ion-exchange monolithic stationary phases for CapIC [6]. Zakaria et al. recently described the use of latex-coated monolithic polymeric stationary phases for the separation of inorganic anions [7]. The column used was a 250 μm I.D. fused silica capillary, with a length of 30 cm, and containing a monolithic stationary phase prepared through the polymerisation of butylmethacrylate and ethylenedimethacrylate with 2-acrylamido-2-methyl-1-propanesulphonic acid, followed by coating with quaternary ammonium latex particles. Separation efficiencies achieved were relatively poor (e.g. 13,000 N/m for iodate), but the separations shown were obtained at high eluent flow rates (up to 31 μL/min, owing to the high porosity of the monolith employed) and so rapid separations could be achieved, with seven analytes resolved in less than 2 min. Most recently, Suzuki et al. [8] have used monolithic silica capillary columns (200 mm × 0.1 mm I.D.) modified with cetyltrimethylammonium salts for the separation of common inorganic anions, including bromide in a seawater matrix. Five anions were separated in under 1 min using a flow rate of just 11 μL/min, with direct UV absorbance detection. However, direct modification of the bare silica monolith with the above surfactant proved unstable over time, requiring addition of the modifier to the eluent to stabilise retention times.

The separation efficiency in microfluidic separations depends strongly on extra-column broadening effects, which are primarily related to the construction of the detector flow cell or suppressor volume for suppressed conductivity detection [7]. On-capillary detection using contactless conductivity detection eliminates these extra-column contributions, and has been extensively utilised for detection in capillary electrophoresis [9]. In their comprehensive review, Kuban and Dasgupta specifically comment on the potential of capacitively coupled contactless conductivity detector (C^4D) as a future detection method for CapIC, and review the application of the technique for use with open tubular capillary columns [3]. However, apart from the above work by Zakaria et al., very little work has yet to emerge in this specific area, with most papers coupling C^4D with capillary electrochromatographic (CEC) separations, such as that carried out by Hilder et al. [10], who used on-column column detection on a packed bed ion-exchange capillary column of 75 μm I.D.

In the following paper we describe for the first time the use of silica based monolithic C_{18} capillary column (150 mm × 0.1 mm I.D.) coated with the amphoteric surfactant DDMAU, in combination with direct-on-column C^4D detection for the rapid CapIC separation and detection of small inorganic anions. The on-column mode of detection was also compared with on-capillary detection using an open tubular capillary to evaluate if measurement across the monolithic bed was significant in relation to detector linearity, sensitivity, etc. The efficiency of the developed method was fully examined and compared to previously published CapIC methods.

2. Experimental

2.1. Instrumentation

The pump used for eluent delivery was an Applied Biosystems 400 Solvent Delivery System (Foster City, CA, U.S.A.). Eluent flow through the capillary column was controlled by a custom built adjustable flow splitter based upon a T-piece connector with variable backpressure applied to the waste line. Flow rates were calibrated using weight of collected eluate in sealed micro-vials. Samples were injected using a Rheodyne MX Module Nano Injector (Alltech Associates, Applied Science Ltd., Lancashire, U.K.), with a fixed injection volume of 10 nL, into which the capillary column itself was connected directly. The capillary column used for this section of work was an Onyx monolithic reversed-phase C_{18} column (150 mm × 0.1 mm I.D., 0.365 mm O.D.) (Phenomenex, Cheshire, U.K.). In a manner similar to that described previously [11,12], the capillary column was dynamically semi-permanently coated with the anion exchanger, *N*-dodecyl-*N*,*N*-(dimethylammonio)undecanoate (DDMAU) (Calbiochem, La Jolla, CA, U.S.A.) by passing a 2.0 mM DDMAU solution through the column at a flow rate of approx. 1 μL/min for 3 h, before washing thoroughly with Milli-Q water for approx. 1.5 h at the same flow rate. Following conditioning the column coating remained stable for the entire period of this study, without signs of column bleed.

The detector used was a TraceDec Contactless Conductivity Detector (Innovative Sensor Technologies GmbH, Innsbruck, Austria). The detector was supplied with a detector cell, through which the above modified monolithic capillary column was directly fed, with the exact position of detection along the length of the capillary variable. Detector settings used were; frequency: 2XH1GH; voltage (amplifier): −18 dB; gain: 75% and offset: 0. Processing of chromatograms was carried out using a PeakNet 6.30 software (Dionex, Sunnyvale, CA, U.S.A.). Eluent pH was measured using an Orion Model 420 pH meter (Thermo Orion, Beverly, MA, U.S.A.) with a glass electrode.

2.2. Reagents

All chemicals used were of analytical reagent grade, and were supplied by Sigma–Aldrich (Tallaght, Dublin, Ireland). All eluents and standard solutions were prepared using deionised water from a Millipore Milli-Q water purification system (Bedford, MA, U.S.A.), and were twice filtered through a 0.45 μm filter and degassed by sonication. The cation-exchange cartridges used were Supelco Supelclean LC-SCX 1 mL tubes (Sigma–Aldrich, Tallaght, Dublin, Ireland).

3. Results and discussion

The reversed-phase monolithic silica based capillary column was dynamically modified with the amphoteric surfactant DDMAU, to provide anion-exchange properties to the monolithic silica phase. This coating has been characterised for its selectivity towards anions previously and has been shown to result in a stable coating on a similar reversed-phase monolithic silica phase [11]. However, it should be noted that according to manufacturer's data on the Onyx monolithic C_{18} capillary column, the capillary monolith has a higher carbon content of 18% mass, compared to the previously studied standard bore Chromolith Performance RP18e column, which has a 14% carbon mass. This should further increase the stability of the surface coating of DDMAU of the capillary monolithic column.

3.1. Column performance

The efficiency of the monolithic anion exchanger with on-column detection was evaluated under varying eluent flow rates. Due to the fact that the monolithic capillary column was connected directly into the injector valve and used with on-column detection, there were no additional connections within the instrumental set-up to produce extra-column broadening, this being an important consideration when reporting efficiency data for such small I.D. columns [13]. A van Deemter plot was constructed for nitrate and iodide peaks (0.5 and 0.25 mM, respectively, 10 nL inj.) with eluent flow rates ranging from 40 nL/min to 1 μL/min ($n = 15$), with an effective column length of 8 cm. The plot is shown as Fig. 1. From the data shown, *A*-, *B*- and *C*-terms were calculated as 20.8 μm, 4.4×10^{-3} mm^2/s and 5.3 ms for nitrate, respectively, and 12.3 μm, 4.0×10^{-3} mm^2/s and 3.1 ms for iodide, respectively (data calculated using Sigmaplot 7 software). The results showed that over the flow rates investigated, peak efficiency was highest between 0.3 and 0.45 μL/min. The obtained *C*-terms between 3 and 6 ms are in a good agreement with data of a recent review [14] which presented *C*-term values for neutral organic compounds on reversed-phase silica based 0.1 mm I.D. capillary monolithic columns, as varying from 2.2 to 70 ms. The *C*-term calculated from the data of Pyo and Kim [5] obtained for an open capillary of 50 μm I.D., coated with latex particles at 75 °C was 57.5 ms, which is ten times higher than the values found in this study (Fig. 1).

The low *C*-term values determined present the possibility to apply higher flow rates through the capillary column without significantly affecting peak broadening. For nitrate, under optimal flow conditions peak efficiency was ~33,500 N/m, and for iodide this value was ~56,200 N/m. These values are similar to those previously observed on the 100 mm × 4.6 mm Chromolith Performance RP18e column coated with DDMAU (39,650 and 49,270 N/m for nitrate and iodide, respectively) [11]. Using a flow rate of 1 μL/min, an average column efficiency was calculated for eight anions (iodate, bromate, nitrite, bromide, nitrate, iodide, sulphate and thiocyanate), using three effective column lengths (L_{eff}) of 4.5, 8.5 and 12.5 cm, and was found to be 35,000 N/m. The observed column efficiency of the monolithic anion exchanger is over 3 times higher that reported by other research groups evaluating different types of capillary ion-exchange columns (Table 1) including organopolymeric monoliths and cetyltrimethylammonium chloride (CTAC) coated monolithic silica CapIC columns [7,8].

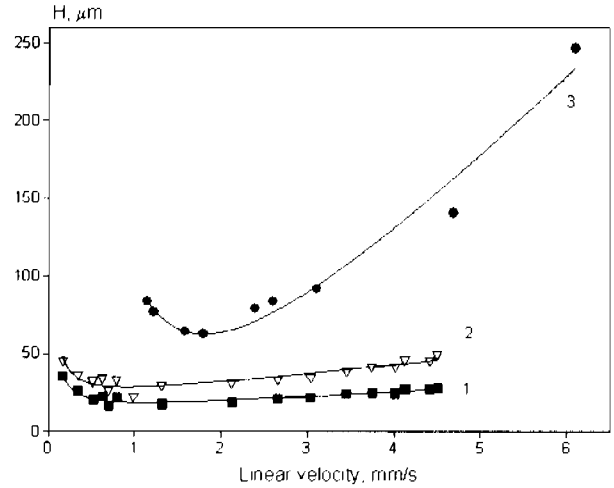

Fig. 1. van Deemter plots for the modified monolithic silica capillary column with an effective column length of 8 cm and a 0.5 mM phthalate eluent (1 and 2) and for the open capillary column 1 m length and 50 μm I.D. coated with latex particles and 1 mM sodium sulphate at 75 °C (3) according to [5]; 1, iodide; 2 and 3, nitrate.

However, the observed values of column efficiency are still less than could be expected for capillary monolithic silica based columns, so it is important to identify the possible reasons for band broadening within the CapIC system. For this purpose, the peak widths (w_h) were measured at different L_{eff} and the corresponding plot was built (Fig. 2). As can be seen from Fig. 2, a continuous increase in peak width was noted for chromatographic peaks of iodide, sulphate and thiocyanate. According to theory the number of theoretical plates in the column (N) can be calculated as follows:

$$N = 16\left(\frac{t_R}{w_h}\right)^2 \quad (1)$$

where t_R is the retention time. Correspondingly, the peak width (w_h) can be expressed as:

$$w_h = 4\frac{t_R}{\sqrt{N}} \quad (2)$$

Assuming the capillary column monolith is homogeneous in its properties, a constant linear velocity ($u = L_{eff}/t_R$) and constant height equivalent to a theoretical plate (HETP = L_{eff}/N) should

Table 1
The column efficiency obtained under optimum conditions for the separation inorganic anions with different capillary columns

Capillary column	Length (mm)	I.D. (μm)	Anion	t_R (min)	Flow rate (μL/min)	Velocity (mm/s)	N/column	N/m	Ref.
Packed with 10 μm agglomerated anion exchanger	470	190	NO_3^-	10.59	1.9	0.67	1,880–3,133	4,000–6,667	[2,3]
Packed with 13 μm IonPack AS11 particles	500	180	Cl	10.82	1.5	–	13,565	27,130	[4]
Open tubular column with internal wall anion exchange coating	5000	75	NO_3	29.8	1.5	5.88	2,763	553	[3]
	1000	50	NO_3	No data	0.177	1.503	20,400[a]	20,400[a]	[5]
AS10 latex coated monolithic methacrylate column	300	250	IO_3	1.566	~8.0	~2.3	3,900	13,000	[7]
Monolithic silica based dynamically coated with CTAC	400	100	NO_3^-	18.59	5.6	–	2,224	5,560[b]	[8]
Onyx monolithic column C18 coated with DDMAU	125[c]/150	100	I	8.92	1.67	0.70	7,025[c]	56,200	Present work

[a] Achieved with decreased viscosity of the eluent at 75 °C.
[b] Calculated from corresponding chromatograms.
[c] Length to the detection point.

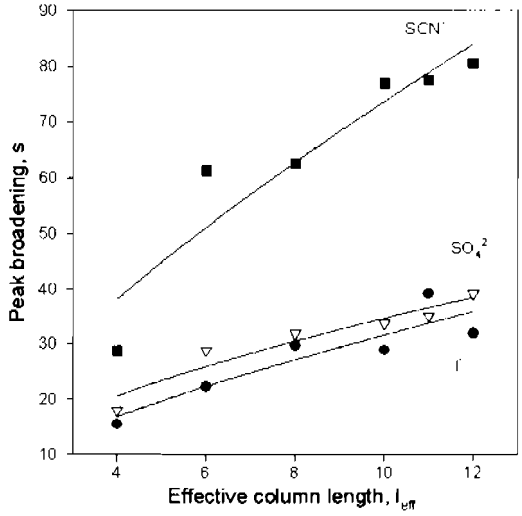

Fig. 2. Graphs showing peak widths (w_b) for peaks of iodide, sulphate and thyocyanate measured at different column lengths (L_{eff}).

be observed at different points of the column or at different L_{eff}. By inserting u and HETP in Eq. (2), the final expression for w_b can be obtained:

$$w_b = 4\frac{L_{eff}}{u}\sqrt{\frac{HETP}{L_{eff}}} = \text{const}\sqrt{L_{eff}} \quad (3)$$

where $4\sqrt{HETP}/u = \text{const}$. This means that the dependence between w_b and L_{eff} should be suited to function $y = ax^b$. In this work b must be around 0.5. The regression analysis of plots shown in Fig. 2 using Sigmaplot 7 software gave the following equations:

SCN^- : $y = 14.097x^{0.7173}$ ($R = 0.9403$) Std. error for $b - 0.1536$
SO_4^{2-} : $y = 9.4018x^{0.5650}$ ($R = 0.9548$) Std. error for $b - 0.0948$
I^- : $y = 6.5767x^{0.6805}$ ($R = 0.9046$) Std. error for $b - 0.1859$

The values of coefficient b are in a good agreement with theory. The relatively high standard errors obtained for regressions can be attributed to uncertainty of the bandwidth determination at the top of the monolithic capillary column. The column volume V (mL) is equal to:

$$V\,(\text{mL}) = \varepsilon L S = 0.25\pi L(\text{I.D.})^2, \quad (4)$$

where ε is the porosity, L the length of monolithic column (cm) and S is the open capillary tube cross section (cm^2). According to manufacturer's data the I.D. of the capillary monolithic column is 0.01 cm and porosity is ~80%. The injection volume was always 10 nL or 10^{-5} mL, so the bandwidth of unretained solute (w_{0b}) at the top of the capillary monolithic column was approximately 0.159 cm. This corresponds to an injection volume of 25 μL into a standard HPLC column of size 250 mm × 4.6 mm, and would therefore suggest injection volume in this case is not the cause of significant band broadening.

3.2. Anion separations

The anion-exchange selectivity of the monolithic C_{18} capillary column coated with DDMAU was evaluated using a 0.5 mM phthalate eluent. Table 2 lists the retention factors for a wide range of anions on capillary column under these conditions, together with some retention data from the previous study using the DDMAU coated Chromolith Performance RP18e column standard bore 2.5 cm × 0.46 cm column. For the anions listed very similar retention data and selectivity compared to the chloride, which was least retained under the conditions used, were noted.

Fig. 3 shows some typical chromatograms of nine inorganic anions in 30 min. The column capacity was also checked with

Table 2
Retention factors and selectivity for separation of anions on the Onyx monolithic C_{18} capillary column coated with DDMAU using a 0.5 mM phthalate eluent as compared with literature data for Chromolith Performance RP18e column [11]

Anion	Onyx monolithic C_{18} capillary		Chromolith performance RP18e	
	Retention factor, k	Selectivity, α_{An/Cl^-}	Retention factor, k	Selectivity, $\alpha_{An/Cl}$
Bicarbonate	0.30	0.291	-	-
Selenite	0.49	0.476		-
Iodate	0.71	0.689	-	-
Formate	0.77	0.748	-	
Arsenate	0.85	0.825	-	-
Bromate	1.00	0.971	-	-
Chloride	1.03	1.000	1.14	1.000
Nitrite	1.17	1.136	-	-
Chloroacetate	1.23	1.194	-	
Chlorite	1.32	1.282	-	-
Bromide	1.39	1.350	-	-
Nitrate	1.52	1.476	2.41	2.114
Chlorate	2.25	2.185	-	-
Iodide	5.35	5.194	6.27	5.500
Sulphite	7.09	6.884	8.09	7.096
Sulphate	7.18	6.971	8.00	7.018
Thiosulphate	13.12	12.738	-	-
Thiocyanate	20.88	20.272	18.36	16.105
Perchlorate	23.40	22.7184	-	

regard to increasing standard concentrations. Fig. 4 shows the overlaid chromatograms obtained for a series of mixed standards of iodate, nitrite, bromide and nitrate. As can be seen from the chromatograms shown, peak broadening for the higher concentration standards indicated that for future work the capacity of the monolith should ideally be increased.

The ability to move the detection point on the capillary when using C^4D detection provides additional possibilities for optimisation of the separation. Fig. 5(a) shows three overlaid chromatograms of the same 0.5 mM mixed standard recorded with different L_{eff}. Fig. 5(b) simply shows the expanded first 3 min section of each chromatogram for clarity. Using the 12.5 cm monolith it was possible to separate all of the anions injected, although the selectivity of the stationary phase coating was such that thiocyanate was retained for ~23 min. However, a considerable advantage of C^4D on-column detection is the ability to alter the L_{eff}, which as shown can mean it is possible, using a single isocratically delivered eluent, to obtain separation of both early and late eluting species, or if it is only the later eluting peaks which are of interest, shorten L_{eff} considerably.

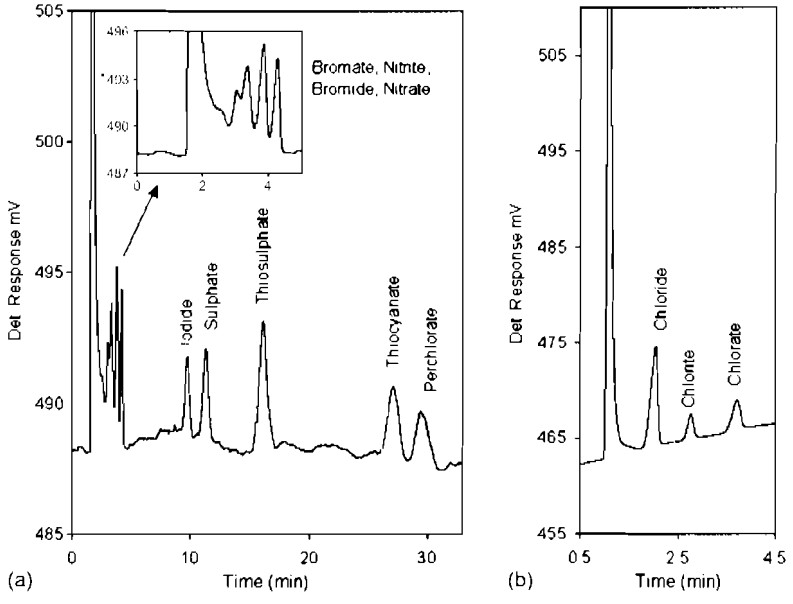

Fig. 3. (a) Typical chromatogram obtained using the modified monolithic capillary column with on-column C^4D detection. Anion concentration = 0.5 mM (except thiocyanate and perchlorate = 2 mM). Effective column length = 8 cm, flow rate = 0.30 μL/min, eluent = 0.5 mM phthalate (pH 4.0). (b) Rapid separation of chloride (0.5 mM), chlorite (2.0 mM) and chlorate (0.5 mM). Conditions as (a).

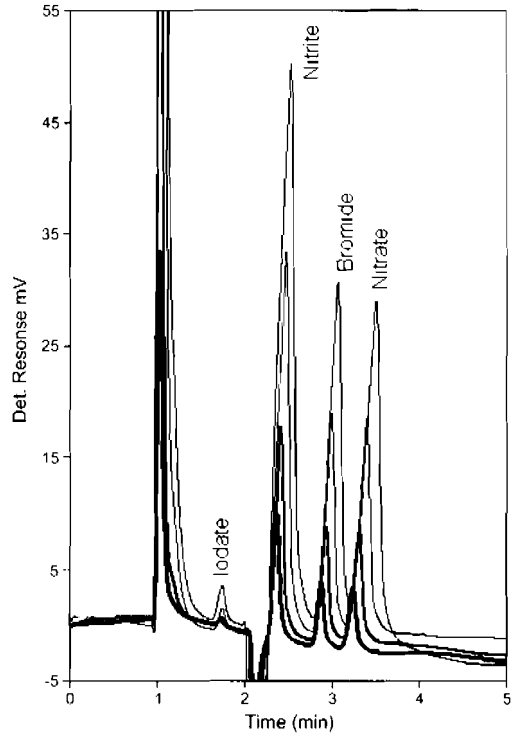

Fig. 4. Overlaid chromatograms of mixed anion standards ranging from 10 to 100 mg/L (100 pg to 1 ng injected mass) obtained using the modified monolithic silica capillary column with a 0.5 mM phthalate eluent (pH 4.0) and on-column C^4D detection. Flow rate = 1.0 μL/min.

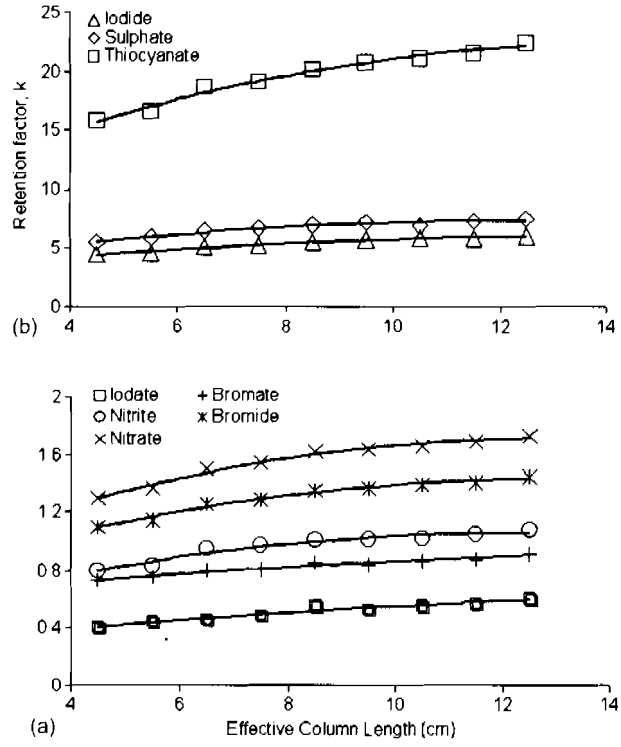

Fig. 6. Graphs showing the change in retention factor, k, as effective column length was increased for (a) iodate, bromate, nitrite, bromide and nitrate, and (b) iodide, sulphate and thiosulphate. Eluent: 0.5 mM phthalic acid (pH 4.0). Flow rate: 1.0 μL/min.

The ability to move the detector cell along the capillary column also provides a simple method to evaluate the longitudinal homogeneity of the column coating. Calculation of k for standard anion peaks at varying L_{eff}, with all other conditions constant, should result in a constant k for each anion. A plot of L_{eff} against k should therefore give a flat response. However, here, as shown in Fig. 6, such a response was not seen, indicating the degree of DDMAU coating was not uniform along the length of the column. The data shown in Fig. 6 would indicate a lower degree of stationary phase coverage at the start of the capillary, as k values increase steadily as L_{eff} increases from 4 to 13 cm. This would indicate a small degree of 'bleed' of the surfactant from the capillary column over time, which would increasingly result in this uneven distribution of the coating over this period. This simple

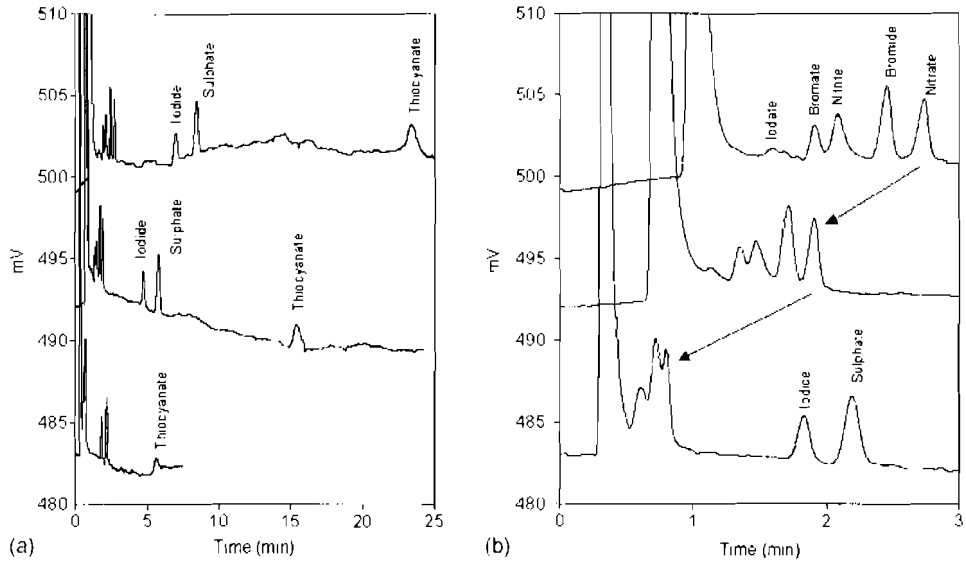

Fig. 5. (a) Capillary ion chromatograms of a 0.5 mM mixed anion standard obtained using the modified monolithic capillary column with on-column C^4D detection. Effective column length = 4.5 cm (bottom), 8.5 cm (middle) and 12.5 cm (top). Flow rate = 1.0 μL/min, eluent = 0.5 mM phthalate (pH 4.0). (b) Expanded first 3 min of above.

method, which is an additional benefit of on-column detection, can be used to improve stationary phase modification methods to achieve a more homogenous distribution of the modifier, and a recently published communication has explored this possibility further [15]. From the experimental point of view, the use of C^4D in this way is a simpler way to evaluate longitudinal uniformity along a capillary, compared with alternative methods, such as laser fluorescence detection in transparent capillary columns as proposed by Evans and McGuffin [16]. From the data shown in Fig. 6 it is clear that more detailed studies into coating procedures and column conditioning is required to achieve a uniform and stable coating along the length of the capillary and these studies are currently underway.

3.3. On-column conductivity detection

To evaluate the suitability of the C^4D detector for use in CapIC as an on-column detector it was important to evaluate detector performance, both directly on the monolithic silica column and also, for comparison purposes, on open tubular fused silica capillary of similar dimensions (100 mm × 0.1 mm I.D.). Taking a 1 mM acetate buffer solution as a model eluent, the detector response for the unmodified monolithic capillary column to 10 nL injections of chloride standards under simple flow injection analysis (FIA) conditions was evaluated (0.28–7.05 mM, 10–250 mg/L). The same series of injections were made onto the open tubular fused silica capillary column, and again detected on-column. For all experiments flow rate through each capillary was kept constant at 0.96 μL/min ± <5%. The resultant calibration graphs for these FIA experiments are shown as Fig. 7, here with data plotted as peak areas. Each data point shown is the average from three replicate injections. The recorded data are plotted without background subtraction and clearly show different detector slopes for each experiment, with the open tubular capillary column showing the larger slope and greater sensitivity.

The porosity of the unmodified monolithic capillary column is about 80% of the total volume of corresponding empty capillary, so the lower conductivity response for each solution could be expected in this case, although the slope for the open capillary column was approximately two times higher than for the monolithic column. However, when comparing background signals for various eluents, including water, within the open capillary and a DDMAU modified monolith, the modified monolith now exhibited a slightly higher background signal due to the contribution of the coating itself, which will obviously negatively affect signal-to-noise ratio when used as here for on-column detection in CapIC. Following the above study, a ~3 cm long section of the 100 μm I.D. open tubular capillary was attached (with zero dead volume capillary connection) to the end of the modified 15 cm long monolithic capillary column and used as a 'detector cell' to compare peak width and height, to those seen with direct detection on the monolith. Fig. 8 shows the resultant chromatograms. The L_{eff} for the direct detection mode (Fig. 8(a)) was ~13 cm, whereas for the monolithic column with attached extension (Fig. 8(b)) this was ~16 cm. As can be seen from the chromatograms shown, the addition of the open capillary tube

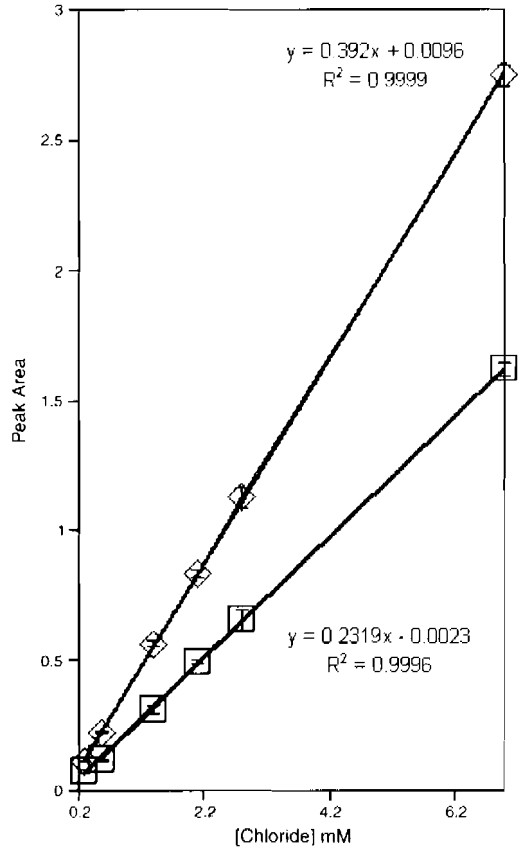

Fig. 7. Peak areas of unretained chloride peaks recorded for the unmodified monolithic capillary column (□) and a 100 μm I.D. open tubular fused silica capillary (◇).

as detector cell led to a clear peak broadening effect due to both increased retention, and more importantly the relatively large internal volume of the open capillary compared to the internal volume of the monolith. Fig. 8(c) shows this effect most clearly with comparison of unretained injection peaks for the two configurations. This comparison indicated that despite the relative responses shown in Fig. 7, the most suitable option for detection was direct detection on the monolithic capillary column itself. Attempts to reduce the band broadening seen through the coupling of smaller I.D. open capillaries (75 μm and 50 μm I.D.) proved equally unsatisfactory as detector response for smaller I.D. capillaries was reduced. Evaluation of the two traces shown in Fig. 8 for comparative levels of detector noise also showed no significant differences.

A series of mixed anion standards (0.06–2.2 mM, $n = 4$) were injected into the monolithic capillary column, and separated using a phthalate eluent. Using on-column detection and recording peak areas, acceptably linear responses were seen for each anion, the slopes and linear regression correlation coefficients for which are given in Table 3. Also shown in Table 3 are peak areas and signal-to-noise ratios for anion standards using direct on-column detection, together with concentration and absolute detection limits (based upon a signal equal to 3 × baseline noise). As shown in Table 3, absolute detection limits based upon the 10 nL injection volume ranged from just 10 pg (bromide) to 115 pg (thiosulphate), corresponding to concentration detection

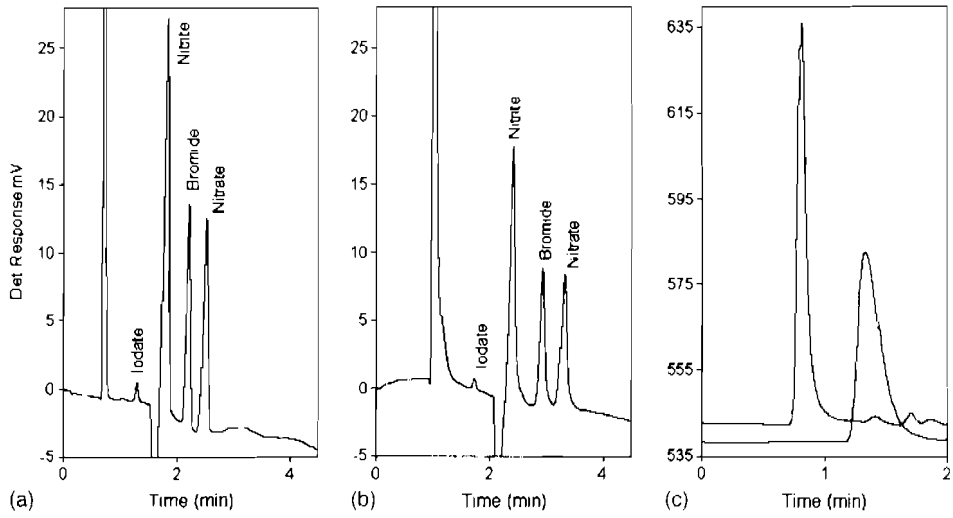

Fig. 8. Separation of four anions on the modified monolithic silica capillary column recorded (a) directly on the monolithic capillary using C^4D at ~13 cm column length, and (b) on an end-coupled 100 μm I.D. open capillary at ~16 cm column length. (c) Comparison of unretained injection peaks recorded with the monolithic capillary column (t_0 = 0.85 min) and with the coupled monolithic and open capillary columns (t_0 = 1.3 min).

limits of 0.012 and 0.073 mM, respectively. These values compare well to that recently published by Suzuki et al. [8] who used UV absorbance detection with monolithic CapIC and achieved detection limits for bromide of 0.056 mM (absolute detection limit of 90 pg using a 20 nL injection volume).

3.4. Analysis of drinking water samples

To assess the suitability of the simple CapIC system with on-capillary detection to a common application, drinking water samples were analysed. The effective column length was set as short as possible (~4.5 cm), in order to give the shortest run times possible. A tap water sample taken from a source tap within the research laboratory was filtered using a 0.45 μm filter, and injected onto the capillary column, without subjecting the water sample to any further sample pre-treatment. The resulting chromatogram, as well as a chromatogram of a 0.5 mM standard of chloride, nitrate and sulphate, for comparative purposes, can be seen in Fig. 9(a). The overall runtime was under 3 min, but there was excessive tailing of the injection peak which partially masked the peaks for chloride and nitrate. This tailing may have been caused by a degree of retention of organic acids within the sample, or slight retention of divalent cations present in the tap water sample, due to the amphoteric nature of the stationary phase coating. The use of a reversed-phase clean-up cartridge to remove organic components from the sample prior to analysis had no effect and so the retention of cationic species was identified as the possible cause.

In an attempt to remedy the above tailing, the samples were injected onto the capillary column through a cation-exchange cartridge in the acid form (Supelco Supelclean LC-SCX). This removed the various cationic species within the tap water sample and replaced them with hydronium ions. While a certain degree of injection peak tailing was still evident, the signal-to-noise ratios for chloride, nitrate and sulphate obtained after performing cation-exchange on the tap water sample were 2.6, 1.9 and 1.5 times the values of the signal-to-noise ratios calculated for the tap water sample that had not been subject to any sample pre-treatment. Fig. 9(b) shows a rapid separation of chloride, nitrate and sulphate in a tap water sample, injected through the cation-exchange cartridge, with an effective column length of ~4.5 cm. Here the three major anionic components of drinking water can be clearly seen with an overall run time of under 1.5 min. Increasing the column effective length to 8.5 cm, whilst still injecting

Table 3
Detector sensitivity and linearity (0.06–2.2 mM, n = 4) for common anions using a 0.5 mM phthalate eluent (pH 4.0), delivered at a flow rate of 1.0 μL/min, with on-column detection

Anion	Peak area (mV min)[a]	Signal-to-noise ratio[a]	Detection limit (3 × noise) (mM)	Absolute detection limit (pg)	Calibration slope	Correlation coefficient (R^2)
Iodate	0.0578	38.3	0.039	68	0.004	0.9902
Bromate	0.1416	67.6	0.022	28	–	–
Nitrite	0.2357	29.5	0.051	24	0.097	0.9954
Bromide	0.4104	123.4	0.012	10	0.052	0.9989
Nitrate	0.3264	106.8	0.014	9	0.059	0.9996
Iodide	0.3706	48.4	0.031	79	0.026	0.9998
Sulphate	0.9149	24.2	0.062	60	0.044	0.9987
Thiosulphate	0.9727	81.7	0.073	115	–	–

[a] For mixed 0.5 mM standard (thiosulphate 2 mM).

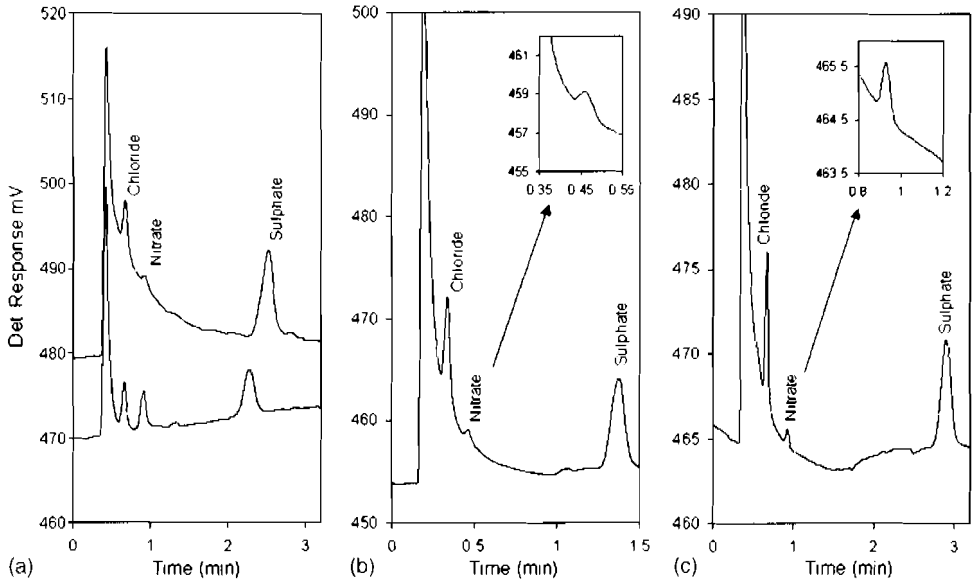

Fig. 9. (a) Overlaid capillary ion chromatograms with on-column C^4D detection of a chloride, nitrate and sulphate 0.5 mM standard solution and an untreated drinking water sample. (b) A capillary ion chromatogram of a drinking water sample following passage through a cation-exchange cartridge (effective column length = ~4.5 cm). (c) As (b) except effective column length = 8.5 cm. Eluent and flow rates same as Fig. 5.

via the cation-exchange cartridge resulted in the chromatogram shown as Fig. 9(c). Clearly resolution of the peaks was improved and the system was more suitable for quantification of the nitrate peak.

Finally, in order to investigate the robustness of the developed method, the analysis of a single tap water sample was repeated 8 times (including the pre-treatment step). Peak area %RSD values of 3.18, 2.72 and 2.81 for chloride, nitrate and sulphate, respectively, were obtained. As the repeat experiments included the pre-treatment step and considering the small sample volumes involved in CapIC, %RSD of ~3% and below for the method was encouraging.

4. Conclusions

The reversed-phase monolithic silica based capillary column coated with the amphoteric surfactant DDMAU was evaluated for the separation of inorganic anions. The direct insertion of the capillary column into the injection valve in combination with on-column detection provided the unique possibility to avoid extra-column peak broadening and to calculate the true values of the *C*-term from the van Deemter plot. The observed separation efficiency is at least twice greater than that previously shown for other monolithic columns used in CapIC.

The possibility of using of on-column contactless conductivity detection was demonstrated here for the first time in CapIC using the modified silica monolithic capillary columns. The system demonstrated here uses a low conducting organic acid eluent, allowing detection and quantification of common anions over the approximate concentration range 1–50 mg/L, based upon a fixed 10 nL injection volume. Under these conditions, using peak areas, detector response was shown to be acceptably linear and so application to simple sample matrices, such as drinking water, was clearly possible. The ability to move the detector cell along the length of the capillary column also meant that run times could be reduced to a point of acceptable resolution simply through reduction in apparent column length, shown in this work with the 1.5 min run time for the determination of chloride, nitrate and sulphate in a drinking water sample.

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